In order for a claimed invention to be obvious, the invention as a whole must be considered, and in particular every limitation of the claim must be disclosed or suggested by the prior art. This means that for the present claims, the cited publications must disclose or suggest a method of transplanting hematopoietic cells from a donor source into a genetically unrelated recipient comprising administering to the recipient, in combination with the administration of the hematopoietic cells, an amount of mononuclear cells which are treated so as to substantially reduce their ability to cause graft versus host disease while they retain their ability to proliferate in the recipient and facilitate engraftment of the hematopoietic cells in the recipient; and administering to the recipient an effective amount of hematopoietic cells. Applicants submit that the cited publications do not disclose or suggest all of these features.

It has been recognized that T cells have both positive and negative effects when present in bone marrow transplant material. On the one hand, T cells are important for efficient engraftment of bone marrow cells in antigenically and genetically mismatched recipients. On the other hand, the presence of T cells in bone marrow transplants increases the incidence of graft versus host disease. In dealing with these effects, those performing bone marrow transplants have tried to balance removal and/or killing of T cells in bone marrow transplant material with retention of T cells in bone marrow transplant materials (Sykes is an example of this). Thus, the art recognized the presence or absence of T cells as being of significance to bone marrow transplants.

Applicants have discovered that it is not the mere presence or absence of T cells that matters in causing the positive and negative effects of T cells on bone marrow transplants. Specifically, Applicants have discovered that a reduction in the viability of T cells without eliminating or killing the T cells outright (that is, treating the T cells such that they retain their ability to proliferate) results in both the reduction of the negative effects of T cells on bone marrow transplants while retaining the positive effects. The present claims specifically claim treatment of mononuclear cells to obtain these benefits. Applicants submit that the cited

publications do not disclose or suggest the claimed effects, do not disclose or suggest any way to obtain the claimed effects, and thus cannot make the present claims obvious.

Relevant to the present rejection, Sykes describes treatments that do not completely deplete the T cells present. In other words, Sykes teaches **retention** of some T cells. Sykes does not disclose or suggest whether the retained cells have **retained the ability to proliferate**. As far as Sykes discloses, the T cells may be able to proliferate freely or may not be able to proliferate at all. Thus, contrary to assertions in the rejection, Sykes does not disclose or suggest that the T cells retain the ability to proliferate. In this regard, Applicants note that art that encompasses (among other possibilities) a particular feature, but which does not disclose that particular feature, does not put those of skill in the art in possession of that particular feature. For example, art disclosing an alloy comprising some nickel does not disclose or make obvious an alloy comprising enough nickel to give the alloy a particular hardness. Until it is discovered that such an effect is possible and that such an amount of nickel is desirable, this particular alloy is unknown and unobvious to those in the art. The situation here is analogous. It is the Applicants who discovered the importance of the claimed treatment and features. None of the cited publications disclose or suggest treatment to obtain the claimed effects.

As discussed above, neither the Waller nor Sykes disclose or suggest the claimed invention. In fact in the Office Action mailed November 18, 2002, the Examiner conceded that the Waller does not teach that the treated T cells retain their ability to proliferate in the recipient. In the April 9, 2003 Office Action, the Examiner states that

> Sykes et al., teach a method of myeloreductive non-myeloablative treatment with fludarabine, the same type of treatment as [the] claimed invention. Sykes et al., teach that for successful transplantation of hematopoietic cells from donor to recipient, it is essential that after treatment T cells are not completely depleted, thus so called graft-versus-leukemia (GvL) effects of the non-depleted T cells help engraftment of donor hematopoietic cells (see page 10, lines 17-23, page 11, lines 5-25 in particular). Sykes et al., specifically stressed that said

treatment should not completely eliminate T cells (page 16, lines 2-11 in particular).

Applicants respectfully point out that what is important is not whether T cells are present, but whether the T cells proliferate. At best, Sykes is silent as to proliferation. In fact, Waller discloses the "same" treatment[1] as Sykes (that is, treatment with fludarabine) but achieves a result completely different from both the result the rejection asserts for Sykes and the result required by the present claims. More specifically, Waller discloses a method of preventing graft-versus-host disease comprising treatment with fludarabine (see column 3, lines 6-16, which discuss the treatment, and column 4, line 66 through column 5, line 12, for the use of fludarabine, in particular). Furthermore, Waller also teaches that "lymphocytes, and especially T cells, present in the allogeneic bone marrow graft are important to ensure engraftment" (column 1, lines 52-55). Waller goes on to teach that "T cells present in the allogeneic graft also have an important role in eliminiating residual cancer cells in the recipient, a phenomenon termed "graft vs. leukemia effect" (column 1, lines 55-58). However, Waller is clear that the T cells are treated so as to render them **incapable** of proliferation (column 3, lines 6-16, column 5, lines 25-31, and claims 1 and 2).

Thus, both Waller and Sykes use fludarabine to reduce T cell populations. Further, only Waller discloses an effect of this use of fludarabine on the proliferative ability of the treated T cells (the proliferative ability is eliminated). In the face of this, it cannot be said that Sykes discloses (or is even consistent with the possibility) that the cells of Sykes retain the ability to proliferate. Accordingly, it would not be obvious to one of skill in the art to use fludarabine to result in T cells capable of proliferating.

Additionally, while Sykes does disclose that T cells should not be completely depleted, this is not the same as saying that the remaining cells would retain the ability to proliferate nor

[1] It is the same treatment only in the improperly broad sense used in the rejection (i.e. ignoring differences in the method based on the presence or absence of specific effects unappreciated in the cited publications).

that such a characteristic would be desirable. The presence or absence of T cells in the recipient is completely independent of their ability to proliferate. The art is replete with examples of non-proliferating T cells (see, for example, Jenkins MK, Schwartz, RH. (1987) *J. Exp Med.* 165:302-19; Jenkins MK, et al. (1987) *Proc Natl. Acad. Sci.* 84:5409-13; Quill H, Schwartz, RH. (1987) *J. Immnuol.* 138:3704-12; abstracts of which are submitted with this Response). Applicants respectfully contend that the Examiner is extrapolating an effect that is not discussed anywhere in Sykes.

Furthermore, the Examiner appears to be under the impression that any treatment with fludarabine would result in proliferating T cells--since that is what is presently claimed--and that Sykes intended that the T cells proliferate. This is incorrect. In fact, and to the contrary, Sykes discloses that "in preferred embodiments, immune cell activity, e.g., T cell activity, preferably graft reactive T cell activity, is inhibited in the subject" (page 14, lines 26-31). By this, Sykes means that the number of T cells is reduced. This is made clear where Sykes defines the term "immune cell activity" as "reducing the **number** of active immune cells, e.g., thymocytes, T cells...in a subject. Inhibition can include partial inhibition or partial reduction (as opposed to total elimination) of the number of active immune cells, e.g., T cells" (page 10, lines 18-22; emphasis added). This definition emphasizes reduction in the number of cells, not in any change in cell characteristics. Thus it is clear that Sykes viewed treatment with fludarabine as a means to reduce the T cell population not maintain the proliferative capacity of the T cells. This view of Sykes is further supported by embodiments that disclose "immunosuppression regimen for suppressing or depleting T cells in the transplanted donor stem cells" (page 5, lines 21-23, page 5, lines 31-33, page 21, lines 6-7, and page 21, lines 16-17), and by the statement (on page 2 lines 15-20) "[l]ikewise, the method can include the further step of treating the subject with an immunosuppressant regimen, after introduction of the donor stem cells.....[s]uch immunosuppressants can include independently of pre- and post-transplantation is [sic] both are carried out, a treatment of the subject which inactivates and/or depletes host T lymphocytes." If the goal, as indicated, is depletion, then surely the depleted cells can not be expected to

proliferate. Furthermore, it is clear throughout the specification and at least on page 15, lines 24-32, that in addition to donor derived T cells, host T cells are also to be depleted. Thus, it is clear that Sykes does not disclose or suggest the use of fludarabine to enable T cells to proliferate, but to the contrary discusses fludarabine only in the context of immunosuppression. For at least these reasons, the combination of Waller with Sykes does not make the claims obvious.

Moreover, it is clear that not all fludarabine treatments would result in a reduced T cell population that retains its proliferative capacity. The art is replete with examples of treatments with fludarabine that resulted in nonproliferative T cells. Waller, discussed earlier, is an example. Numerous publications in the area use fludarabine to eliminate T cells. Goodman et al., (1996) *Am. Surg.* 62(6):435-442 (the abstract submitted with this Response), states that "[f]ludarabine phosphate selectively eliminates normal and malignant mononuclear cells in large animals and man." Additionally Goodman et al. report that "[t]he drug depletes mononuclear cells from culture within 24 hours of initial exposure, CD4 and CD8 T cells being more sensitive than either CD20 B cells or CD34 bone marrow precursors." Additionally, Boulad et al., (2000) *Br. J. Haematol.* 111(4):1153-7 (abstract submitted with this Response), discusses fludarabine-based cytoreductive treatment in a subject with Fanconi anaemia. Contemporary with Sykes et al. and Waller, the art of hematopoietic stem cell transfers was filled with publications detailing the importance of reducing or depleting T cell populations to prevent graft versus host disease, not retaining T cells (see for example; Link, (1999) *Baillieres Best Pract Res Clin Haematol.* 12(1-2):87-98 (abstract submitted with this response), and Slaper-Cortenbach, ICM, et al., (1999) *Rheumatology* 38:751-754 (copy submitted with this response)). For at least these reasons, the combination of Waller with Sykes does not make claims obvious.

Lastly, the Examiner supports his position with the paragraph on page 10, lines 17-23, of Sykes, which discusses the definition of "inhibiting immune cell activity," Referring, in particular, to the last sentence which states "[i]nhibition can include partial inhibition or partial reduction (as opposed to total elimination) of the number of active immune cells e.g., T cells." The Examiner reads this to mean that total elimination is not desired (and thus, impliedly, that

proliferation is desirable). However, the more reasonable reading of this passage is not that total elimination is undesirable, but rather a recognition that a small residual population of T cells would likely remain following treatment and therefore the Sykes specification was written to reflect that T cells may remain after treatment. This passage does not refer to the **proliferative** capacity of the T cells. As such, applicants submit that this passage does not support the Examiner's position.

Applicants believe that the rejection has been overcome and respectfully request that it be withdrawn. In the event that the Examiner does not find the arguments presented herein to be persuasive, applicants respectfully request that the examiner address why each point is not valid or deemed persuasive. To this end, and to further prosecution, Applicants request a telephonic interview with the Examiner.

Pursuant to the above remarks, reconsideration and allowance of the pending application is believed to be warranted. The Examiner is invited and encouraged to directly contact the undersigned if such contact may enhance the efficient prosecution of this application to issue.

A Credit Card Payment Form PTO-2038 authorizing payment in the amount of $55.00, representing the fee for a small entity under 37 C.F.R. § 1.17(a)(1) is enclosed. This amount is believed to be correct; however, the Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-0629.

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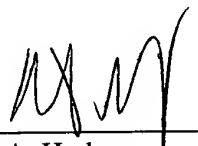
NEEDLE & ROSENBERG, P.C.



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The depletion of T cells from haematopoietic stem cell transplants

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Abstract

Objective. In our laboratory, we have developed an immunorosette technique for the depletion of T cells from bone marrow transplants. Tetrameric complexes of monoclonal antibodies are able to form very stable immunorosettes, which are efficiently depleted with the aid of a blood cell separator. Major improvements over the original sheep red blood cell depletion are the use of human (patient or donor derived) erythrocytes instead of sheep-derived cells, and the possibility of using a closed system for separation in a cell separator. In contrast to bone marrow, mobilized haematopoietic stem cell transplants obtained after leucocytapheresis contain higher numbers of T cells. Therefore, a different approach is necessary.

Method. We have used two CD34 selection systems (Isolex® 300SA and the Clinimacs®) to perform T-cell depletions from peripheral blood stem cell (PBSC) transplants.

Results. Immunorosette T-cell depletion, with CD2/CD3 tetrameric complexes, of bone marrow transplants resulted in a mean 2.5 log depletion of T cells with a yield of 50% of the $CD34^+$ cell population. Stem cell selection of PBSC transplants using one of the CD34 selection procedures resulted in a 4.5 log depletion of T cells for both systems, but with different results for the recovery of $CD34^+$ cells. An increased yield of $CD34^+$ cells was obtained with the Clinimacs® procedure (57.9 ± 9.0%) in comparison to the Isolex® procedure (40.1 ± 12.5%).

Conclusion. Our own immunorosette depletion technique and the two tested CD34 selection methods for stem cell transplants both resulted in a very efficient T-cell depletion with the recovery of 40–60% of the $CD34^+$ haematopoietic stem cells present in the transplant.

KEY WORDS: T cells, Depletion, Haematopoietic stem cell transplants.

In the past, several different T-cell depletion techniques have been developed to avoid graft-*vs*-host disease in an allogeneic stem cell transplantation setting. Kernan *et al.* [1] have applied an agglutination technique using soy bean agglutinin (SBA) followed by T-cell depletion using sheep red blood cells (SRBC). This method was the first and one of the most widely applied T-cell depletion techniques for the removal of T cells from bone marrow. Others have performed methods, for instance, based on the difference in cell size between T cells and haematopoietic stem cells, known as the counterflow elutriation technique [2], or antibody-mediated techniques, like complement-mediated cell lysis using Campath antibody [3].

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In our institute, Lansdorp *et al.* [4] developed a technique using bivalent antibody complexes, known as tetrameric complexes. We have modified the application of this method and introduced an immunorosette procedure to couple the patient's own erythrocytes to target cells for efficient depletion. Originally, this technique was set up to deplete malignant cells from patients with B-cell malignancies [5], but we have also produced tetrameric complexes for the removal of T cells. This technique can now be used to deplete T cells from allogeneic or autologous bone marrow stem cell transplants.

Since peripheral blood stem cells (PBSC) are rapidly replacing bone marrow as a source of haematopoietic stem cells, other techniques have been developed for the removal of T cells. PBSC transplants from normal donors contain higher percentages of T cells, and often >10 times the total number of T cells. This necessitates another approach. CD34 cell selection is an efficient method to achieve T-cell removal. Several different methods for clinical application are commercially available with variable results. Cellpro was the first on the market with a Food and Drug Administration-approved column system (Ceprate^{R} SC) using biotin-labelled CD34 monoclonal antibody (MAb) in combination with avidin-coated Sepharose beads. This selection procedure results in a 40–50% yield of CD34 stem cells with a purity of $\sim$80%. Then, Baxter introduced the Isolex$^{®}$ system for positive selection, using Dynabeads^{R} to isolate the stem cells and a CD34-releasing peptide (PR 34+$^{®}$) to dissolve the bond between the stem cells and the beads. This method also results in a 40–50% yield of CD34^{+} cells, but with a much higher purity of $>90\%$ CD34^{+} cells. Recently, Miltenyi Biotec has introduced an immunomagnetic system, Clinimacs$^{®}$, using very small beads (colloidal super paramagnetic MicroBeads), which can efficiently enrich the stem cell population ($>90\%$ pure) like the Isolex$^{®}$ system, but with a higher CD34 yield of $\pm$ 65% [6]. Using this system, the beads, which are very small, are not removed from the surface of the CD34^{+} stem cells.

Autologous stem cell transplants have recently been introduced for the treatment of patients with autoimmune diseases [7]. Most of these diseases are believed to be T-cell mediated, so a depletion of autologous T cells seems indicated. However, an exact T-cell dose is not yet known, but a more extensive reduction than 1×10^{5} T cells/kg body weight (BW) might not be necessary (van Bekkum [8]).

In this article, we present an outline of our results obtained with immunorosettes for the depletion of T cells from bone marrow, and CD34 selection for the depletion of T cells from PBSC.

Materials and methods

Allogeneic stem cell transplants

Patients suffering from several different haematological malignancies, who have an allogeneic donor available, were treated with high-dose chemotherapy and total body irradiation. On the day of transplant, bone marrow cells were taken from either HLA-identical siblings or matched unrelated donors, and sent to the cell-processing laboratories of the CLB in Amsterdam, or of the Academic Hospital in Utrecht. More recently, the use of PBSC transplants for rapid haematopoietic reconstitution in an HLA-identical setting has become more beneficial for the patients than using bone marrow as a source of stem cells. Donors were given 5 μg granulocyte-colony stimulating factor (G-CSF) for mobilization of PBSC. The leucocytapheresis procedures were performed at the Academic Medical Centre. The PBSC transplants were transported to the CLB and either CD34 selected directly or the next day. After selection, the cells were taken to the hospital and directly infused.

Autologous stem cell transplants

Autologous bone marrow transplants were harvested from children with juvenile chronic arthritis, according to a protocol described in this issue by Wulffraat and Kuis [9].

Techniques for T-cell depletion

Immunorosette depletion of bone marrow. Tetrameric complexes are formed by the addition of cross-linking RaMIgG1 MAb to a mixture of MAbs, one directed against glycophorin A in the membrane of human erythrocytes and another T-cell-specific MAb (CD2 or CD3). These complexes are then bound to erythrocytes and the coated erythrocytes are washed. The bone marrow harvest is centrifuged, prior to depletion, and a buffy coat suspension is prepared to get rid of the excess erythrocytes. After addition of the coated erythrocytes to the bone marrow buffy coat cells, immunorosettes are formed. These immunorosettes are depleted using a Ficoll density separation ($d = 1.077$ g/cm^{3}) in an IBM 2991 cell processor and the light density cells are washed and cryopreserved.

CD34 selection of PBSC. In our laboratory, we have used two different CD34 selection systems for the removal of T cells from PBSC transplants: Isolex$^{®}$ 300SA (Baxter Biotech group, USA) and the Clinimacs$^{®}$ (Miltenyi Biotec, Germany). Procedures were performed according to the manufacturers' instructions.

Quality control

The quality of the transplant was measured using the myeloid progenitor cell assay (CFU-GM, colony forming unit for granulocytes and monocytes) with human placental conditioned medium as a source of growth factors, and by monitoring the CD34 content. The efficacy of the T-cell depletion technique was measured either by means of the immunorosette method or by immunofluorescence using the FACscan flow cytometer (Becton Dickinson, Mountain View, CA, USA).

Results

Immunorosette depletion

The immunorosette technique was successfully applied to allogeneic bone marrow transplants from HLA-identical siblings (Table 1).

The target number of T cells to be transplanted at the Academic Medical Centre was set at 1×10^5 T cells/kg BW, according to the protocol described by Verdonck *et al.* [10]. This protocol aims to minimize the risk of graft-*vs*-host disease and inducing a graft-*vs*-leukaemia effect in patients with haematological malignancies.

In all CD2/CD3-depleted stem cell transplants, a supplement of unseparated bone marrow cells was necessary to reach 1×10^5 T cells/kg BW. This enabled transplantation of exact numbers of T cells in these patients. In four patients, the recovery of the CD34 haematopoietic stem cell population was measured and the CD34 yield was 49.1 ± 18.8%.

Furthermore, we have used the same technique to reduce the number of T cells in autologous bone marrow transplants from children with juvenile chronic arthritis (Fig. 1). Here, the aim was to reach an even lower level of T cells: 1×10^4 T cells/kg BW. By performing a second round of T-cell depletion, this target number was reached in two out of six autologous transplants, with an average of $5.1 \pm 4.9 \times 10^4$ T cells/kg BW for all six patients.

The mean CD34 recovery was 53.5 ± 27.5%, which allowed the infusion of $1.5 \pm 1.1 \times 10^6$ $CD34^+$ cells/kg BW (range 0.5–3.6).

In Utrecht, the same CD2 and CD3 immunorosette procedure was performed on 34 allogeneic bone marrow transplants, resulting in a 2.3 log depletion of T cells (Fig. 2).

CD34 selection

For the depletion of T cells from PBSC transplants, we have used two different CD34 selection systems: the Isolex® and Clinimacs®. The average depletions of T cells from PBSC transplants for both procedures are equal: 4.5 logs (Fig. 2). However, there is a great difference in the recovery of $CD34^+$ cells: the Isolex® procedure resulted in a median recovery of 40.1 ± 12.5%, while the Clinimacs® procedure resulted in a 57.9 ± 9.0% recovery of $CD34^+$ cells.

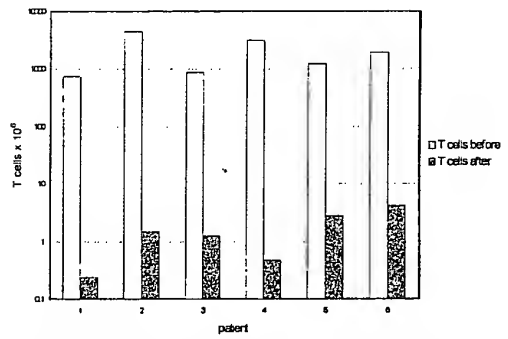

FIG. 1. Results of the depletion of CD2 and CD3 immunorosetted T cells from bone marrow transplants of children with juvenile chronic arthritis. Depicted are the total T-cell numbers in the bone marrow harvest (T cells before) and total T-cell numbers after depletion.

Discussion

The main advantage of using the immunorosette depletion technique instead of the SBA/SRBC procedure is the fact that it can be performed in a closed system. Very stable immunorosettes are formed, which can easily be separated in the IBM cell processor. Furthermore, binding of the patient's own or donor-derived erythrocytes to the T cells circumvents the use of SRBC, which cannot be produced according to good manufacturing practice regulations. In our procedures, MAbs were used that were screened according to the CLB Biosafety Testing protocols, in the absence of bacterial and viral contamination. Moreover, combining the removal of immunorosettes within one round of density separation

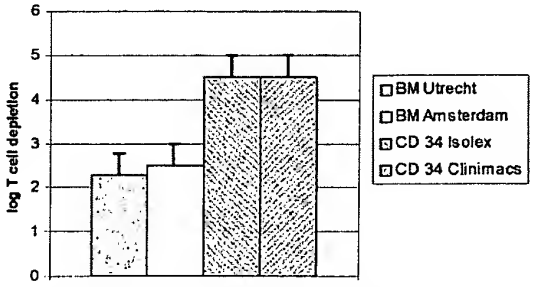

FIG. 2. T-cell depletion results of bone marrow and PBSC transplants obtained with the different methods. Bone marrow processing was performed at two centres.

TABLE 1. Results of the T-cell reduction in bone marrow transplants of HLA-identical donors.

	Cell number $\times 10^8$	CFU-GM $\times 10^4$	$CD3^+$ cells $\times 10^6$
Bone marrow harvest ($n = 10$)	176.8 ± 45.3	1910 ± 749	2769 ± 719
CD2/CD3 depleted ($n = 10$)	10.19 ± 4.27 (5.8 ± 2.4%)	780 ± 419 (50.9 ± 19.4%)	0.55 ± 0.90 (0.022 ± 0.045%)

The recovery of each cell population is given in parentheses.

reduced the processing time from 9 to 6 h, thereby saving ~50% of the $CD34^+$ cells. Using immunorosettes for the depletion of T cells from bone marrow, a significant removal of T cells occurred, averaging a 2.5 log T-cell depletion.

So far, we have only limited experience with the isolation of $CD34^+$ cells from bone marrow ($n = 2$), with varying results: 6% yield with the Isolex® system and 50.2% with the Clinimacs. Apart from the prolonged selection procedure (density separation followed by selection), the CD34 selection methods are all very expensive. This method is not only costly because of the separation device, but also because the disposables, media and CD34 kits are expensive.

For PBSC transplants, however, we have so far tested both immunomagnetic systems of Baxter and Miltenyi. In a recently published study [11], the CD34 selection systems of Cellpro and Baxter were compared, resulting in a 3.4 median log T-cell depletion for the Isolex® 300I system and a 2.9 log T-cell depletion for the Ceprate^R system.

Our own results indicate that similar T-cell depletion efficacies are being reached using the two immunomagnetic selection systems Isolex® 300SA (Baxter) and the Clinimacs® (Miltenyi).

In our laboratory, we are currently performing experiments to develop new methods using the tetrameric complexes for depletion of larger numbers of T cells in combination with a nylon wool filtration technique as described in part by Kwekkeboom *et al.* [12] so that we can perform a much cheaper technique than the CD34 selection for PBSC.

Moreover, we think that CD34 selection is not the optimal method for T-cell depletion, since all other cell types are also excluded from the transplant, including cells which, in an allogeneic setting, might play a role in the engraftment of the haematopoietic stem cells. Further studies will indicate whether we can successfully develop a T-cell depletion technique for PBSC transplants.

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Molecular events in the induction of a nonresponsive state in interleukin 2-producing helper T-lymphocyte clones.

Jenkins MK, Pardoll DM, Mizuguchi J, Chused TM, Schwartz RH.

Exposure of normal interleukin 2 (IL-2)-producing helper T-cell clones to antigen and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide-treated antigen-presenting cells results in proliferative unresponsiveness to subsequent stimulation with antigen and normal antigen-presenting cells. In the present study, we have examined the molecular events that accompany the induction of this unresponsive state. T cells stimulated in this manner failed to produce IL-2, but interleukin 3, interferon-gamma, and IL-2 receptors were partially induced and T-cell receptor beta mRNA was fully induced. Although T-cell unresponsiveness correlated with an IL-2 production defect, addition of IL-2 during the induction phase failed to prevent development of the unresponsive state. The critical biochemical event appeared to be an increase in intracellular calcium. Removal of calcium from the medium prevented induction of the unresponsive state, whereas addition of the calcium ionophore ionomycin induced unresponsiveness as well as all of the related partial activation events. Thus, an increase in intracellular calcium under nonmitogenic conditions appears to initiate an alternative activation program that prevents the T cell from producing IL-2 in response to subsequent normal activation signals. The significance of this in vitro model for tolerance induction in vivo is discussed.

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Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness in vitro and in vivo.

Jenkins MK, Schwartz RH.

We investigated the antigen specificity and presentation requirements for inactivation of T lymphocytes in vitro and in vivo. In vitro studies revealed that splenocytes treated with the crosslinker 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (ECDI) and soluble antigen fragments failed to stimulate significant proliferation by normal pigeon cytochrome c-specific T cell clones, suggesting that the chemical treatment inactivated full antigen presentation function. However, T cell clones exposed to ECDI-treated splenocytes and antigen in vitro were rendered unresponsive for at least 8 d to subsequent antigen stimulation with normal presenting cells. As predicted by the in vitro results, specific T cell unresponsiveness was also induced in vivo in B10.A mice injected intravenously with B10.A, but not B10.A(4R), splenocytes coupled with pigeon cytochrome c via ECDI. The antigen and MHC specificity of the induction of this T cell unresponsiveness in vitro and in vivo was identical to that required for T cell activation. These results suggest that nonmitogenic T cell recognition of antigen/MHC on ECDI-modified APCs results in the functional inactivation of T cell clones.

PMID: 3029267 [PubMed - indexed for MEDLINE]

1: J Immunol 1987 Jun 1;138(11):3704-12 Related Articles, Links

Stimulation of normal inducer T cell clones with antigen presented by purified Ia molecules in planar lipid membranes: specific induction of a long-lived state of proliferative nonresponsiveness.

Quill H, Schwartz RH.

Culture of normal inducer T cell clones with antigen and purified Ek beta:Ek alpha incorporated into planar lipid membranes resulted in specific T cell activation as determined by cell volume increase and IL 3 production. However, in contrast to results obtained with T cell hybridomas, antigen presentation by planar membranes did not induce measurable IL 2 production, and proliferative responses were not detected. Rather, recognition of only Ek beta:Ek alpha and antigen resulted in the specific induction of a long-lived state of proliferative nonresponsiveness to subsequent stimulation by conventional APC and antigen. Induction of nonresponsiveness required protein synthesis, and was not simply due to the absence of IL 2. The antigen-nonresponsive cells could respond to either PMA plus ionomycin or IL 2, and they expressed normal levels of surface antigen-receptor molecules. These results demonstrate that recognition by normal T cell clones of antigen and Ia molecules in the absence of other accessory cell molecules and signals results in a prolonged state of proliferative nonresponsiveness, possibly similar to a state of T cell tolerance in vivo.

PMID: 3035012 [PubMed - indexed for MEDLINE]

1: Am Surg. 1996 Jun;62(6):435-42. Related Articles, Lin

Fludarabine phosphate: A DNA synthesis inhibitor with potent immunosuppressive activity and minimal clinical toxicity.

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Fludarabine phosphate selectively eliminates normal and malignant mononuclear cells in large animals and man through the inhibition of DNA synthesis. The drug depletes mononuclear cells from culture within 24 hours of initial exposure, CD4 and CD8 T cells being more sensitive than either CD20 B cells or CD34 bone marrow precursors. Mitogenic activation of lymphocytes enhances cellular elimination from culture. Fludarabine inhibits PHA-induced T-cell proliferation by >90 per cent and mixed lymphocyte reactions (allogeneic and xenogeneic) by >95 per cent. Fludarabine exerts its cytolytic effects through the induction of endonuclease-independent apoptosi A 5-day course of fludarabine (50 mg/m2 intravenously once daily) induces both T- and B-cell lymphopenia in Cynomolgus monkeys and Papio baboons Transient neutropenia was the only side-effect seen in experimental animals. Pretreatment of Cynomolgus monkeys with this regimen of fludarabine caus a prolongation of ABO-compatible skin allograft survival from 8 days (control) to 16 days (drug treated group). Secondary allotransplantation into presensitized recipients showed a similar prolongation of graft survival with fludarabine pretreatment (8 days vs 5 days control). Fludarabine promises to be a potent immunosuppressive agent with low clinical toxicity.

PMID: 8651523 [PubMed - indexed for MEDLINE]

1: Br J Haematol. 2000 Dec;111(4):1153-7. Related Articles, Lin

Stem cell transplantation for the treatment of Fanconi anaemia using a fludarabine-based cytoreductive regimen and T-cell-depleted related HLA-mismatched peripheral blood stem cell grafts.

Boulad F, Gillio A, Small TN, George D, Prasad V, Torok-Castanza J, Regan AD, Collins N, Auerbach AD, Kernan NA, O'Reilly RJ.

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We have employed a new cytoreductive regimen to transplant two patients with Fanconi anaemia (FA), using T cell-depleted two HLA-allele disparate related peripheral blood stem cell transplants (PBSCTs). Patient 1, a 5-year-old male with FA and aplastic anaemia, initially received an HLA two-antige mismatched unrelated cord blood transplant and failed to engraft. He receive fludarabine (Flu) and cyclophosphamide (Cy), followed by a CD34(+) E-rosette(-) (CD34(+)E(-)), T cell-depleted, granulocyte colony-stimulating factor (G-CSF)-mobilized PBSCT from his HLA B-DRB1 mismatched fathe He received anti-thymocyte globulin (ATG), steroids, FK506 and G-CSF aft transplant for rejection and graft-versus-host disease (GVHD) prophylaxis. The patient is now 23 months after SCT with no evidence of GVHD and witl full haematopoietic and immune reconstitution. Patient 2, a 10-year-old boy with FA and myelodysplastic syndrome, received single-dose total body irradiation (SDTBI), Flu and Cy followed by a CD34(+)E(-), T-cell-depleted G-CSF-mobilized PBSCT from his HLA B-DRB1 mismatched sister. He als received ATG, steroids, FK506 and G-CSF after transplant. The patient is now 12 months after SCT in complete remission with no evidence of GVHD Absolute neutrophil counts (ANC) of > 1 x 10(9)/l were achieved on day 11 and day 10 post transplant respectively. Both patients are fully engrafted. In summary, we report two successful T-cell-depleted stem cell transplants fron mismatched related donors for the treatment of Fanconi anaemia, using a fludarabine-based cytoreduction. Both patients experienced minimal toxicity. rapid engraftment and no GVHD.

PMID: 11167755 [PubMed - indexed for MEDLINE]

1: Transfusion. 2000 Dec;40(12):1475-81. Related Articles, Lin

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Isolation and flow cytometric analysis of T-cell-depleted CD34+ PBPCs.

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BACKGROUND: To extend allogeneic HPC transplantation to a greater range of patients, the use of partially matched related donors is under development. Because of the inherently higher degree of histoincompatibility in such transplants, there is increased risk of GVHD as well as of graft failur Ex vivo depletion of donor-derived T-lymphocytes from PBPCs is one of the most effective methods of preventing GVHD. Thus far, individual centers have used custom-developed procedures to deplete the graft of T cells that ar responsible for alloreactivity, often employing relatively impure, nonstandardized reagents such as soybean agglutinin and complement. In addition, with improved methods of T-cell depletion, it has been difficult to accurately assess the number of T cells remaining. Because different centers have used different protocols to assay T cells, it has been difficult to reproduce and validate the results between institutions, and this has limited direct comparison of data between centers. STUDY DESIGN AND METHODS: A standardized approach for T-cell depletion was developed by using a Good Manufacturing Practice-manufactured magnetic cell separator (Isolex 300i, Nexell Therapeutics) and commercially available OKT3 antibody. T-cell depletion was performed on PBPCs from six haploidentical donors. RESULTS: CD34+ cell recovery was 47 percent (range, 31-63%) with a median purity of 94 percent (range, 75-99%) and median T-cell log depletion of 4.72 (range, 3.90-5.83). Because this high degree of depletion makes it challenging to accurately quantitate the remaining T cells, two highl sensitive flow cytometric protocols were developed, each of which accurately detects T cells with a sensitivity of 2 per 10,000 (0.02%). The purified CD34 cells administered to the patients (dose range, 6.13-13.50 x 10(6)/kg) provide rapid neutrophil and platelet engraftment. CONCLUSION: With the Isolex 300i and a MoAb directed against T cells, a high degree of T-cell depletion i obtained. Sensitive, accurate, and reproducible assays have now been developed for T-cell enumeration in these highly purified cell populations.

1: Baillieres Best Pract Res Clin Haematol. 1999 Mar-Jun;12(1-2):87-98.

T-cell depletion of allogeneic peripheral blood stem cells.

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The high content of immunocompetent T-cells in apheresis products may expose recipients of allogeneic peripheral blood stem cells (PBSC) to an elevated risk of acute and chronic graft-versus-host disease (GvHD). Thus, th use of an appropriate T-cell reduction or depletion technique might reduce th risk. The hazards of rejection and of a higher relapse rate should be avoided by maintaining a portion of the T-cells in the graft or by increasing the number of transplanted stem cells. The positive selection of CD34+ cells fro peripheral blood preparations simultaneously provides an approximately 1,000-fold reduction of T-cells. Purified CD34+ cells containing committed and pluripotent stem cells are suitable for allogeneic transplantation. In transplantation from HLA-mismatched or three HLA-loci different family donors the amount of stem cells can be increased for reducing the incidence rejection without increasing the T-cell number. In cases of poor marrow graf function a 'boost' with stem cells from the same family donor can be given. The risk of GvHD in transplantation from volunteer-matched unrelated dono might be reduced by T-cell depletion. If T-cells are used for enhancing the graft-versus-leukaemia effect, CD34+ enriched cells can be given for haematopoietic engraftment.

Publication Types:
- Review
- Review, Tutorial

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